

# Surface-modified reusable gold electrode for detection of dissolved oxygen

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**Abstract** The behavior of gold electrodes for the detection of dissolved oxygen was studied by the method of cyclic voltammetry in a phosphate-buffered solution with physiological pH. Surface modification with electropolymerized poly (*o*-phenylenediamine) film was performed to improve electrode antifouling properties. The voltammetric signature of oxygen was considered in terms of film electropolymerization conditions and post-deposition conditioning of the electrodes. The changes in the chemical structure of the poly (*o*-phenylenediamine) films as a result of these factors were confirmed by X-ray photoelectron spectroscopy analysis. Following long post-deposition conditioning in a phosphate-buffered solution, the modified electrodes exhibited stable voltammetric signatures in repeated tests and during storage as well as in the presence of a dense population of *Escherichia coli* (characterized by negligible metabolic activity) in the buffer. The results are indicative of the improved electrode antifouling properties.

**Keywords** Electropolymerization · Poly (*o*-phenylenediamine) · Electrode modification · Electrode stability · Oxygen detection · Antifouling

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## 1 Introduction

Monitoring of dissolved oxygen in biological systems can provide important information about their vital activity [1–3]. In many cases, such sensors should be small in size, flexible (allowing easy insertion and extraction), mechanically stable and biocompatible. The development of miniature planar electrochemical oxygen sensors on flexible substrates, therefore, holds much interest for biological and medical applications.

Electrochemical amperometric oxygen sensors include an electrochemical cell in which oxygen is reduced on the cathode and an oxygen permeable membrane for separating the analyte and the internal electrolyte and for preventing the fouling of the working electrode [4, 5].

The use of Au thin film electrodes in planar biosensors is preferable because of their biocompatibility and relatively easy and inexpensive fabrication with standard silicon compatible microfabrication methods [6]. However, even the simplest phosphate-buffered solutions (PBS), commonly used for analysis of biological species, tend to foul Au electrodes as a consequence of phosphate anion adsorption on their surface [7–9].

The microfabrication of sensors for the detection of dissolved oxygen presents a difficult technological challenge because the methods used for membrane preparation and internal electrolyte incorporation are typically incompatible with the most common microfabrication techniques [10, 11]. To avoid the process incompatibilities, surface modification was introduced in the design and fabrication of electrochemical sensor electrodes. Polymer-modified electrodes were reported to confer antifouling properties and improved sensor selectivity in the detection of a number of products [12, 13], solving the main problems of electrochemical biosensors operation, namely, electrode

fouling and interferences [4, 5, 10]. The approach of polymer-modified electrodes does not exclude the other approaches for solving these problems, particularly the use of differential-pulse amperometric technique with biocompatible carbon paste electrodes [14]. We adopted modification using electrochemical polymerization, because it is compatible with mainstream silicon manufacturing technology and can be performed in existing fabrication facilities without requiring specialized production lines.

Electropolymerized films for preventing electrode fouling and interferences were studied over many years. However, these works were mainly concentrated on biosensors in which electropolymerized films were used for the modification of anodes [15–22]. Poly (*o*-phenylenediamine), PoPD, films produced by electropolymerization of *o*-phenylenediamine (*o*-PD) are very attractive for application in such biosensors in view of their excellent permselectivity for low molecular weight analyzed species and rejection of large organic molecules.

As the detection of O<sub>2</sub> occurs on the cathode surface, its catalytic activity towards oxygen reduction reaction (ORR) is desirable. Electrocatalysis of oxygen reduction takes place at the cathodes modified by a number of electropolymerized films, particularly by PoPD [23–28]. The catalytic activity of PoPD films towards ORR in acidic solutions was shown; both electropolymerization and oxygen detection were conducted in the Na<sub>2</sub>SO<sub>4</sub> solutions (pH 1.0 adjusted with H<sub>2</sub>SO<sub>4</sub>) [23–25, 28]. However, no data were reported on the behavior of PoPD films at physiological (neutral) pH and on the stability of oxygen reduction signal during the PoPD-modified electrodes storage and operation.

The purpose of this study is to analyse the above issues, which are critical for application of electrochemical biosensors. The behavior of PoPD-modified electrodes was studied in terms of the PoPD films preparation (electrodeposition and post-deposition conditioning), stability and antifouling properties of the films. To understand electrode behavior, the chemical structure of the modifying films was studied by XPS technique in relation to their preparation conditions.

## 2 Experimental

### 2.1 Electrode preparation

The work was conducted on the PoPD-modified Au foil electrodes and on the modified thin film 200 nm Au/20 nm Cr electrodes deposited on polyimide. Thin films were deposited by methods of physical vapor deposition (PVD). Unless otherwise specified, the areas of the foil and thin film electrodes were 0.2 cm<sup>2</sup> and 0.1 cm<sup>2</sup>, respectively. The pretreatment of Au foils for PoPD deposition was as follows: polishing by alumina (Al<sub>2</sub>O<sub>3</sub>, 0.3 μm) slurry, boiling in H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> (volume ratio 3/1) for 1 min and cycling in 0.5 M H<sub>2</sub>SO<sub>4</sub> to obtain a reproducible pattern. Thin film electrodes were modified without any pretreatment.

The Au electrodes surface was modified with PoPD films deposited by oxidative electropolymerization in a phosphate-buffered solution (PBS) containing 30 mM *o*-PD. The PBS composition is shown in Table 1. Electropolymerization was performed under different conditions, Table 2. Preparing the acidic polymerization solution, the pH of the PBS was adjusted to 1 with H<sub>2</sub>SO<sub>4</sub> before addition of *o*-PD.

### 2.2 Measurement of the PoPD films thickness and refractive index

These parameters were determined by means of spectroscopic ellipsometry using a J.A. Woollam M-2000 DUV system with quartz tungsten halogen/deuterium light source. Data were acquired at an angle of 70°, in the wavelengths range of 700–1,000 nm and the Cauchy–Urbach model was used to fit the data.

### 2.3 Electrochemical tests

Detection of dissolved oxygen was studied by the method of cyclic voltammetry (CV). The experiments were conducted in the potential range from +0.1 V to −0.7 V, at a scan rate 50 mV s<sup>−1</sup> in PBS, which is usually used as the medium for biological tests (pH 7).

**Table 1** Compositions of PBS and LB solutions

PBS	Composition (kg m <sup>−3</sup> )	LB	Composition (kg m <sup>−3</sup> )
Sodium chloride (NaCl)	8.0	Sodium chloride (NaCl)	10
Potassium chloride (KCl)	0.2	Yeast extract Difco-Bacto	5
Potassium phosphate, monobasic anhydrous (KH <sub>2</sub> PO <sub>4</sub> )	0.2	D(+)-Glucose	2
Sodium phosphate, dibasic dihydrous (Na <sub>2</sub> HPO <sub>4</sub> 2H <sub>2</sub> O)	5.8	Tryptone. Difco-Bacto	10

**Table 2** Conditions of electropolymerization of *o*-phenylenediamine

Conditions	Solution (pH)	Potential versus SCE (V)	Time (s)
1	7	+0.7	100
2		+0.7	300
3		+0.7	900
4 <sup>a</sup>		+0.7	900
5		+0.7 V for 200 s followed by 25 cycles +0.7 V/−0.7 V	
6		25 cycles +0.7 V/−0.7 V	
7		10 cycles 0.0 V/+0.9 V	
8	1	+0.7	300
9		+0.7	900
10		10 cycles 0.0 V/+0.9 V	

Scan rate was 50 mV s<sup>−1</sup> in all cases of electropolymerization by potential cycling

<sup>a</sup> Electropolymerization solution was deaerated

Electropolymerization and voltammetric tests were conducted in the solutions containing dissolved oxygen in equilibrium with atmosphere unless specified otherwise. The electrode potentials during electropolymerization and voltammetric tests of the electrodes were measured and reported versus the saturated calomel electrode (SCE).

For electropolymerization and electrochemical tests, an ECO CHEMIE Autolab potentiostat was used. Experiments were performed at room temperature in three-electrode cells, employing Au working and Pt counter electrodes. Post-deposition cathodic treatment of the electrodes at −0.7 V for 5–60 min and their long conditioning (up to 50 days) were conducted in PBS at room temperature.

## 2.4 Preparation of *Escherichia coli* cells

*Escherichia coli* cells (strain RFM443) were grown overnight in Luria–Bertani (LB) broth (see Table 1) containing 0.1 mg cm<sup>−3</sup> ampicillin with shaking at 37 °C. The overnight culture was grown to an optical density of 1.87 (600 nm) prior to their use in the experiments. The cells were then centrifuged and the resulting pellets were suspended in PBS buffer to give  $\sim 10^9$  cells cm<sup>−3</sup>.

## 2.5 X-Ray photoelectron spectroscopy (XPS)

XPS measurements were performed in UHV (33.33 nPa base pressure), using 5600 Multi-Technique System (PHI, MN). The samples were irradiated with an Al K<sub>α</sub> monochromated source (1,486.6 eV) and the outcome electrons were analyzed by a Spherical Capacitor Analyzer using a slit aperture of 0.8 mm in diameter. Neutralization of positive charging was used. All peaks were referenced to C

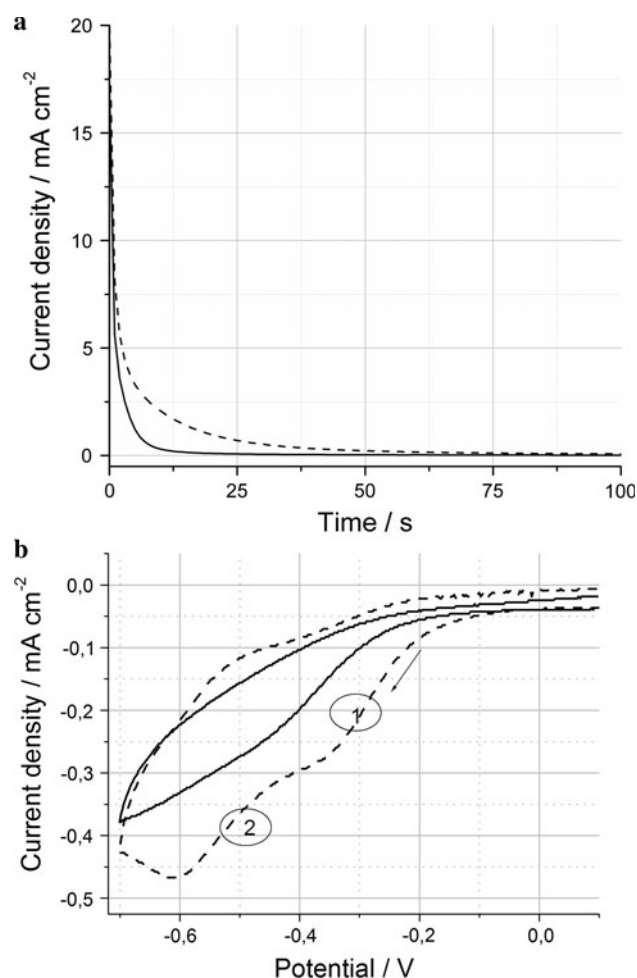
1s peak position at 285 eV. High-resolution measurements were done at a pass energy of 11.75 eV at increments of 0.05 eV step<sup>−1</sup> to allow for precise energy position and peak shape determination. Curve fitting was performed with Gaussian–Lorentzian sum function by using a 5600 Multi-Technique System software. The fitting components were defined in all the samples to have the same values of peak energy position and its full width at half maximum.

## 3 Results and discussion

### 3.1 Modification of electrodes by *o*-PD electropolymerization

Figure 1a demonstrates the anodic current densities versus time for *o*-PD electropolymerization at constant potential. The current density decrease was slower in the case of electropolymerization at pH 1 than in the case of electropolymerization at pH 7. When electropolymerization was conducted by potential cycling (voltammograms not shown here), we observed the same effect of pH, in agreement with results reported in the literature [29–31]. The peak attributed to oxidation of monomer and formation of radical-cation was near +0.6 V for pH 1 and near +0.4 V for pH 7. This peak gradually decreased with increased cycle number at pH 1, while it strongly decreased from the first cycle at pH 7. This is indicative of the quick formation of the film and substantial blocking of the electrode surface. These differences in the electrochemical characteristics of electropolymerization are consistent with the variations in behavior and chemical structure of the formed PoPD films as discussed in the following.

The thickness of the electropolymerized PoPD films showed only a weak dependence on the pH; it was  $19 \pm 5$  nm at pH 7 and  $17 \pm 5$  nm for pH 1. These results are similar to those reported in the literature for the films produced at pH 7 and pH 1; the low thickness of the films can be understood from the point of view of the self-limiting nature of the electropolymerization process [22, 32, 33]. However, higher thickness values were also reported [22, 34]. The thickness of 130 nm was determined for the film deposited for a long time (15 min) from a neutral concentrated (300 mM) solution of the monomer. The overestimated thickness might be a consequence of codeposition of by-products with the film on the cathode of an electrochemical quartz crystal microbalance used for determination [22]. The thickness of the films electropolymerized in 100 mM H<sub>2</sub>SO<sub>4</sub> solution increased with the number of electropolymerization cycle from about 50 nm for 50 cycles to about 830 nm for 450 cycles. No increase of the film thickness was observed with further increase of the number of cycles [34]. Although the nature of



**Fig. 1** The effect of the *o*-PD electropolymerization pH on electropolymerization process and electrode parameters. **a** Current density change during electropolymerization at constant potential, +0.7 V, sample area 0.15 cm<sup>2</sup>. **b** The voltammetric signatures of PoPD as-modified Au thin film electrodes in PBS. The numbers in circles show the waves for the electrode modified at pH 1 *continuous line* electropolymerization at pH 7; *dashed line* electropolymerization at pH 1

electropolymerization process is self-limiting, the relatively high conductivity of the films produced in strong acid solution allows deposition of relatively thick films, when the process is sufficiently long. The data obtained at low cycle number are consistent with our data.

The refractive index of the films produced in our study was in the range of 1.47–1.54, which is somewhat lower than the value of 1.62 (at 800 nm) reported for 23-nm PoPD film electropolymerized on ITO electrode in acid solution [33].

### 3.2 Voltammetric signatures of as-modified PoPD electrodes

Cycling voltammograms of the as-modified PoPD thin film Au electrodes are presented in Fig. 1b. There is significant difference in the voltammetric signatures of the electrodes

modified at different pH levels (conditions 2 and 8, Table 2). The voltammogram obtained for the electrode modified in acidic solution contained two waves while that obtained for the electrode modified at pH 7 contained one wave. When the potential was swept in the cathodic direction, significant (about 0.20 V) depolarization was observed for electrodes modified at pH 1.

The effect of electropolymerization solution pH is significant and is a factor defining the type of voltammetric signatures of the as-deposited films. Varying the electropolymerization conditions at constant pH (e.g., conditions 1–7 and 8–10), signatures of the same types, as shown in Fig. 1b, were observed.

It should be noted that the voltammetric signatures of the films produced under identical conditions may vary to some extent. This is presumably associated with the high sensitivity of the electropolymerization process and structure of the films to the surface conditions of the substrate. However, electrode behavior in consecutive voltammetric tests, in response to the treatment at negative potentials and during long conditioning in PBS, was the same for different electrodes, as for the Au foil and Au thin film electrodes.

### 3.3 Effect of repeated consecutive cyclic voltammetry tests

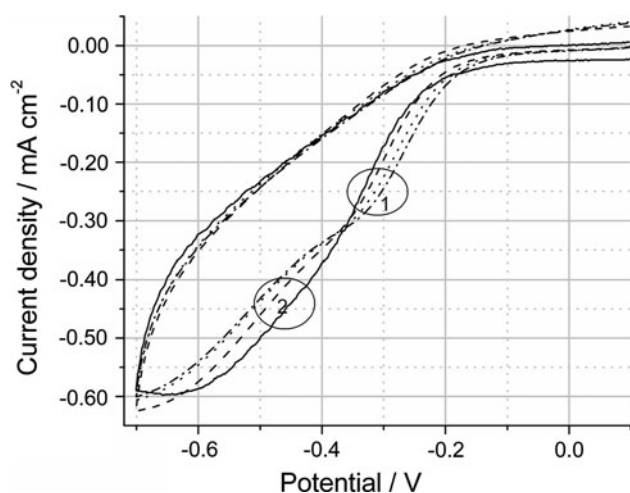
As-modified electrodes were tested repeatedly. To maintain the permanent oxygen concentration, stirring of solution was done before every consecutive test. For the electrodes modified at pH 7, the changes in voltammograms occurring in repeated consecutive cyclic voltammetry tests are similar irrespective of the voltammetric signatures of the as-deposited films. An example is shown in Fig. 2, where the *continuous line* depicts the first test. The following features can be noted:

- The first test is different from the consecutive tests.
- In the consecutive tests, two reduction waves were observed: wave (1), i.e., the upper part and wave (2), i.e., the lower part.
- The shape of the voltammograms obtained on the same day was rather reproducible. However,
- Wave (1) shows some depolarization in consecutive tests.
- Wave (2) shows some polarization in consecutive tests.

The voltammetric signatures of the electrodes modified at pH 1 retained their shape in the repeated consecutive cyclic voltammetry tests, but, wave (1) was slightly depolarized.

### 3.4 Effect of PBS deaeration

Wave (1) in Figs. 1b and 2 should be mainly attributed to ORR as can be seen from the comparison of the tests in



**Fig. 2** Repeated consecutive cyclic voltammetry tests of PoPD-modified Au foil electrode. Electropolymerization by potential cycling (conditions 6, Table 2) *continuous line* the first test, *dashed line* the second test, *dotted line* the fifth test, *dash and single-dot line* the sixth test

aerated and deaerated (by 15 min bubbling of  $N_2$ ) solutions (Fig. 3). The wave (2) is probably associated with the reduction of PoPD itself, although further experiments may be needed to confirm this point.

### 3.5 Conditioning the PoPD-modified electrodes at negative potential

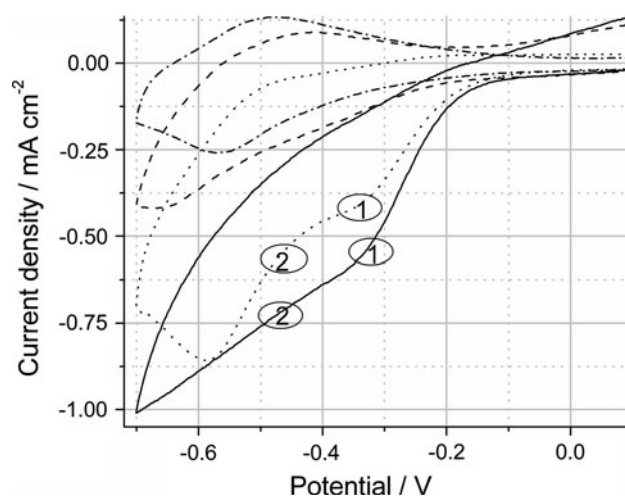
The typical changes in shape of the voltammetric signature of electrode modified at pH 7 as a result of conditioning at  $-0.7$  V are shown in Fig. 4. Conditioning caused the appearance of two waves, instead one, in the voltammograms and a significant depolarization of the wave associated with ORR. Conditioning the electrodes modified at pH 1 at  $-0.7$  V led to the depolarization of the wave associated with ORR and to the increase of observed currents.

The modified electrodes reached a type of saturation after which no additional depolarization with conditioning time was observed. The ORR wave shoulder in voltammograms of saturated electrodes is typically near  $-0.28$  V though shifts up to  $-0.15$  V were observed for some electrodes.

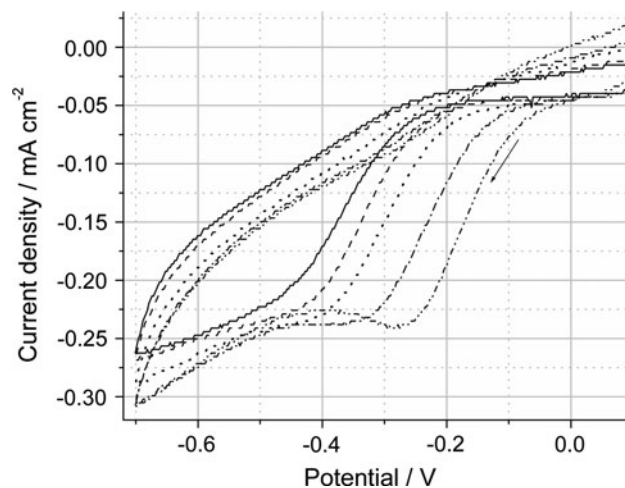
### 3.6 Long conditioning of the PoPD-modified electrodes in PBS

The electrode stability during long storage is very important for realization of the electrode calibration and test reproducibility. Three types of modified electrodes were conditioned in PBS for a long period of time:

- (A) Electrodes that were treated cathodically and reached saturation



**Fig. 3** The effect of deaeration of PBS solution on voltammetric signatures of Au foil electrodes modified by electropolymerization of *o*-PD under different conditions.  $+0.7$  V, pH 7, 100 s (conditions 1, Table 2): *continuous line* before deaeration, *dashed line* after deaeration.  $+0.7$  V, pH 1, 900 s (conditions 9, Table 2): *dotted line* before deaeration, *dash and single-dot line* after deaeration

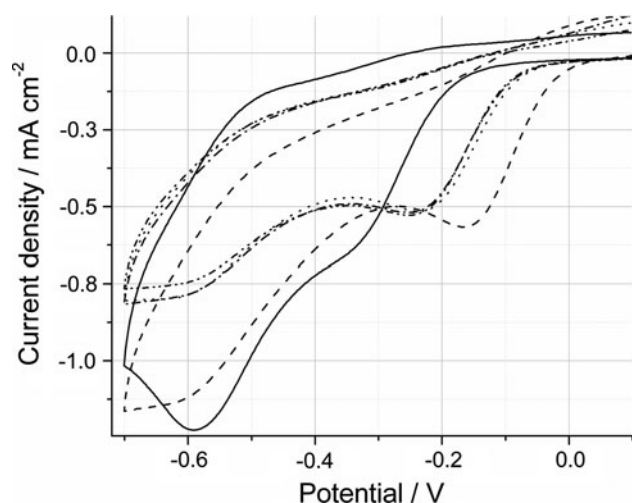


**Fig. 4** The effect of conditioning time at a potential of  $-0.7$  V on voltammetric signature of PoPD-modified thin film Au electrode. Modification conditions:  $+0.7$  V, pH 7, 300 s *continuous line* as deposited; *dashed line* 5 min; *dotted line* 10 min; *dash and single-dot line* 15 min; *dash and double-dot line* 20 min

- (B) Electrodes that were treated cathodically and did not reach saturation
- (C) Electrodes without cathodic treatment

Although the voltammetric signature of the electrodes (A) were well reproduced on the day of cathodic treatment, storage of the treated electrodes in PBS for a few days caused a polarization of the wave associated with ORR and a decrease in its height (Fig. 5). The modified electrodes could be recovered by conditioning at negative potential





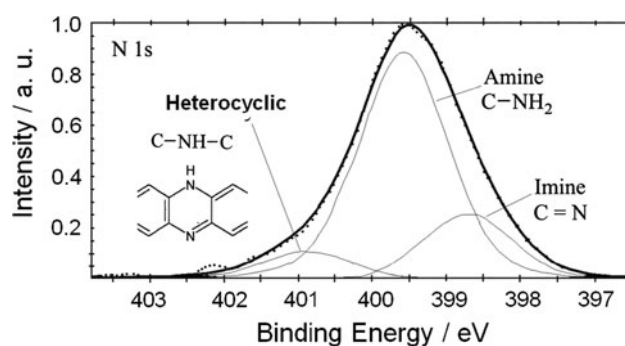
**Fig. 5** The effect of conditioning at a potential of  $-0.7$  V and subsequent long-time conditioning in PBS on the voltammetric signature of PoPD-modified Au foil electrode: Modification conditions:  $+0.7$  V, pH 1, 300 s *continuous line* as-modified; *dashed line* conditioned at  $-0.7$  V, 1,500 s; *dotted line* 12 days; *dash and single-dot line* 17 days; *dash and double-dot line* 30 days. Note that the *three lines* corresponding to 12, 17 and 30 days are essentially identical

( $-0.7$  V). However, the loss of the catalytic activity occurred again. Stabilization of the voltammograms occurred after 10–12 days of storage following cathodic treatment. The stabilized voltammograms were polarized by about 100 mV relative the voltammograms of the electrodes after cathodic treatment.

The changes of voltammograms of the electrodes (B) and (C) were opposite to those occurred during the storage of electrodes (A). Conditioning led to a strong depolarization and an increase of the wave attributed to ORR. Finally, the voltammetric signatures of these electrodes also stabilized as a result of storage in PBS. The final position and height of the analytical wave (1) is rather different for different films and, presumably, depend on their electropolymerization conditions and prior history. However, conditioning of an individual film can endow it with satisfactory stability of the signal associated with ORR.

### 3.7 The behavior of PoPD-modified electrodes in the presence of *Escherichia coli*

The stabilized voltammetric signatures of the PoPD-modified electrodes (after conditioning in PBS) did not change in the presence of a population of bacteria with negligible metabolic activity introduced in the solution in the range from  $\sim 10^7$  to  $\sim 10^8$  cells  $\text{cm}^{-3}$ . No changes were observed both immediately after an addition of the cells in solution nor after 1 h. This result is indicative of



**Fig. 6** The N 1s spectrum curve fitting for Au thin film electrode as-modified by electropolymerization of *o*-PD at  $+0.7$  V and pH 7 for 300 s *dotted line* experimental data; *continuous line (bold)* total fitted curve; *continuous line (thin)* the components obtained from curve fitting

antifouling properties of PoPD-modified electrodes, not only towards PBS but also towards bacteria.

### 3.8 The results of XPS analysis

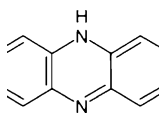
Figure 6 illustrates a typical N 1s spectra curve fitting for as-modified PoPD thin film electrodes, modification performed at pH 7. All fitting results for as-modified, conditioned at  $-0.7$  V and conditioned for a long time in PBS-modified electrodes are summarized in Table 3. The fitted spectra contain three components:

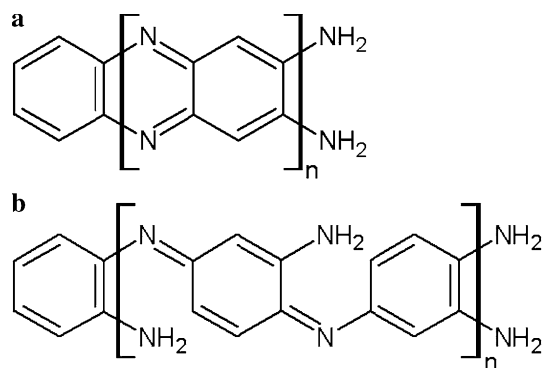
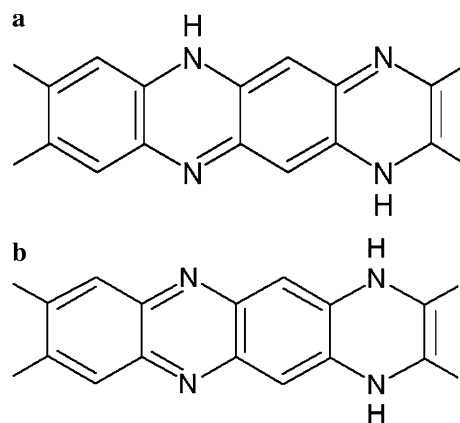
- At about 398.7 eV, attributed to iminic nitrogen of the C=N bonding in the phenazine-like structure (Scheme 1a [35]) [29, 31, 36].
- At about 399.7 eV, related to aminic nitrogen of the C-NH<sub>2</sub> bonding in the benzenoid-quinoid structure (Scheme 1b [34]) [29, 31, 36].
- At about 400.7 eV, assigned to aminic nitrogen of C-NH-C bonding in heterocyclic structures [37–39]. Although the binding energy of this component is in proximity to nitrogen in C=N-OH oxime [29, 37, 40] and NHC=O amide [31, 39] groups, the formation of oxime as well as amide is not likely to occur during electropolymerization process [29]. On the contrary, the formation of heterocyclic structures with C-NH-C bonding (Scheme 2a, b [35]) is well understood from the point of the mechanism of oxidative electropolymerization as a result of the partial anodic oxidation [24, 41]. Therefore, we mainly associate this component with nitrogen of C-NH-C bonding in heterocyclic structures.

A curve-fitting summary, given in Table 3, together with the presented spectra reveals the following effects:

- The concentration of aminic nitrogen ( $58 \div 72$  %) is higher than that of the iminic nitrogen ( $19 \div 27$  %) for all investigated samples. Our results are in a good agreement with the results reported by Losito et al.

**Table 3** Effect of the electrode preparation conditions on the content of different functional groups in modifying PoPD films, as determined from N 1s spectra curve fitting

Electrode preparation	Binding energy of functional group ( $E_b$ , eV) and its content (%)		
	I Imine C=N (398.7 eV)	II Amine C–NH <sub>2</sub> (399.7 eV)	III Heterocyclic C–NH–C  (400.7 eV)
As-modified electrode, modification at pH 7	19	72	9
Electrode modified at pH 7 and conditioned at $-0.7$ V for 900 s	17	72	11
Electrode modified at pH 7 and conditioned in PBS for 7 days	18	62	20
Electrode modified at pH 7, conditioned at $-0.7$ V for 900 s and conditioned in PBS for 7 days	15	62	22
As-modified electrode, modification at pH 1	27	58	15
Electrode modified at pH 1, conditioned at $-0.7$ V for 900 s and conditioned in PBS for 7 days	21	55	24

Modification by electropolymerization at  $+0.7$  V for 300 s**Scheme 1** Chemical structure of **a** poly(*o*-phenylenediamine) phenazine [35] and **b** 1,4-substituted benzenoid-quinoid [34]**Scheme 2** Reduced forms of phenazine [35] **a** asymmetrical quinoid and **b** symmetrical dihydro (1,4-diazine)

[29, 41] and confirm the hypothesis that PoPD films consist of 1,4-substituted benzenoid-quinoid structure units (Scheme 1b) or contain them along with phenazine structure units (Scheme 1a).

- (ii) The concentration of aminic nitrogen in the modifying films deposited at pH 7 is higher than in those deposited at pH 1 (72 vs. 58 %). This is also in agreement with the results of Losito et al. and designates that the films deposited at pH 7 contain low amount of phenazine structure units.
- (iii) The concentration of heterocyclic structures with C–NH–C bonding in the modifying as-deposited film produced at pH 1 is higher than in that produced at pH 7 (15 vs. 9 %).
- (iv) The electrode conditioning at  $-0.7$  V (reduction of the PoPD films) as well as its long conditioning in

PBS led to the decrease of iminic and aminic components and to the increase of concentration of heterocyclic structures with C–NH–C bonding. The latter one increased at the expense of the iminic component following the reduction and mainly at the expense of aminic component as a result of the long conditioning in PBS.

The increase in concentration of heterocyclic structures with C–NH–C bonding as a result of conditioning at  $-0.7$  V is consistent with the reduction of iminic C=N bonds in phenazine structure (Scheme 1a) and with the formation of a reduced form of phenazine structure— asymmetrical quinoid structure (Scheme 2a). The formation of a symmetrical dihydro-compound (Scheme 2b) seems less likely because of its instability [35].

The increase in concentration of heterocyclic structures with C–NH–C bonding following conditioning in PBS is apparently because of conversion of 1,4-substituted benzenoid-quinoid structure through intramolecular cyclization into the structure with reduced phenazine units (Scheme 2a). This is consistent with a decrease of the aminic component. The similar conversion in PoPD films was observed as a result of multiple potential cycling and is believed to occur because of higher thermodynamic stability of phenazine structure units possessing higher conjugation [42]. An asymmetrical quinoid structure has even higher stability than completely conjugated phenazine structure [35]. The decrease of the iminic component during conditioning can be explained by hydrolysis of imine groups with formation of carbonyl (quinone) groups [29, 36, 40].

The results of studying C 1s spectra of modified electrodes (not shown here) confirm the conclusions regarding the structure transformations of PoPD modifying films following electrode conditioning.

Consideration of electrode behavior after cathodic treatment and long conditioning in terms of the PoPD chemical structure allows for the following interpretation of the observed phenomena. The as-deposited PoPD films contain phenazine, cyclic quinoid and 1,4-substituted benzenoid-quinoid units. Concentration of the last units is higher in the films produced by electropolymerization at high pH. These as-deposited films exhibited lower catalytic activity towards ORR than those deposited at low pH. The 1,4-substituted benzenoid-quinoid structure is thermodynamically unstable and transforms into a cyclic quinoid structure during long conditioning in PBS. Cyclic quinoid and dihydro (1,4-diazine) structures can also be formed as a result of phenazine units reduction. These transformations of the chemical structure of PoPD films were accompanied by an increase of the film catalytic activity towards ORR. The films in their saturated condition after long cathodic treatment seem to be unstable (likely because of the instability of the dihydro (1,4-diazine) structure [35]) and transformed during conditioning in PBS into asymmetrical quinoid and phenazine structures. The consequence of these transformations is the decrease of catalytic activity of the films. Thus, the behavior of PoPD-modified electrodes and the results of XPS analysis are consistent with the way of catalysis suggested in literature [23]:

- Formation of symmetrical dihydro (1,4-diazine) structure
- Reaction of 1,4-diazine moiety with O<sub>2</sub> forming adduct
- Rearrangement of adduct
- Formation of H<sub>2</sub>O<sub>2</sub> and phenazine unit

## 4 Conclusions

The PoPD-modified gold electrodes were shown to be suitable for the detection of dissolved oxygen at physiological pH. The voltammetric signatures of modified electrodes in a phosphate-buffered solution and the chemical structures of the modifying films were influenced by the electrode preparation. Based on the results of high-resolution X-ray photoelectron spectroscopy analysis, we believe that low electropolymerization pH and post-deposition cathodic treatment contribute to the formation of phenazine structure units and their reduced forms increasing catalytic activity of the modified electrodes towards oxygen reduction. A long (10–12 day) conditioning of the electrodes in a phosphate-buffered solution stabilized their characteristics and increased their catalytic activity owing to the conversion of 1,4-substituted benzenoid-quinoid units in the modifying films into the more thermodynamically stable asymmetrical quinoid units (reduced form of phenazine units) as a result of intramolecular cyclization. The presence of 10<sup>8</sup> cell cm<sup>-3</sup> *Escherichia coli* (with negligible metabolic activity) in a phosphate-buffered solution did not influence the voltammetric signatures of the stabilized electrodes. As such, it can be concluded that the conditioned PoPD-modified gold electrodes are reusable for repeated tests.

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## References

1. Ivnitski D, Abdel-Hamid I, Atanasov P, Wilkins E (1999) *Biosens Bioelectron* 14:599
2. Yang Z, Sasaki S, Karube I, Suzuki H (1997) *Anal Chim Acta* 357:41
3. Galaction AI, Cascaval D, Oniscu C, Turnea M (2004) *Biochem Eng J* 20:85
4. Clark LC, Wolf R, Granger D, Taylor Z (1953) *J Appl Physiol* 6:189
5. Knake R, Jacquinet P, Hodgson AWE, Hauser PC (2005) *Anal Chim Acta* 549:1
6. Cheng J, Jandik P, Avdalovic N (2003) *Anal Chem* 75:572
7. Weber M, Nart FC, de Moraes IR (1996) *J Phys Chem* 100:19933
8. Niaura G, Gaigalas AK, Vilker VL (1997) *J Phys Chem B* 101:9250
9. Magnussen OM (2002) *Chem Rev* 102:679
10. Suzuki H (2000) *Electroanalysis* 12:703
11. Kudo H, Iguchi S, Yamada T, Kawase T, Saito H, Otsuka K, Mitsubayashi K (2007) *Biomed Microdevices* 9:1
12. Cosnier S (2003) *Anal Bioanal Chem* 377:507
13. Wang J, Golden T (1989) *Anal Chem* 61:1397
14. Lowry JP, Boutelle MG, O'Neill RD, Fillenz M (1996) *Analyst* 121:761
15. Sasso SV, Pierce RJ, Walla IR, Yacynych AM (1990) *Anal Chem* 62:1111



16. Malitesta C, Palmisano F, Torsi L, Zambonin PG (1990) *Anal Chem* 62:2735
17. Geise RJ, Adams JM, Barone NJ, Yacynych AM (1991) *Biosens Bioelectron* 6:151
18. Lowry JP, McAteer K, El Atrash SS, Duff A, O'Neill RD (1994) *Anal Chem* 66:1754
19. Palmisano F, Guerrieri A, Quinto M, Zambonin PO (1995) *Anal Chem* 67:1005
20. Hamdia N, Wang J, Monbouquette HG (2005) *J Electroanal Chem* 581:258
21. Dai YQ, Zhou DM, Shiu KK (2006) *Electrochim Acta* 52:297
22. Killoran SJ, O'Neill RD (2008) *Electrochim Acta* 53:7303
23. Ohsaka T, Watanabe T, Kitamura F, Oyama N, Tokuda K (1991) *J Chem Soc Chem Commun* 16:1072
24. Jang DH, Yoo YS, Oh SM (1995) *Bull Korean Chem Soc* 16:392
25. Premkumar J, Ramaraj R (1996) *J Appl Electrochem* 26:763
26. Khomenko VG, Barsukov VZ, Katashinskii AS (2005) *Electrochim Acta* 50:1675
27. Kumar SA, Chen S-M (2007) *J Mol Catal A Chem* 278:244
28. Gajendran P, Saraswathy R (2007) *J Phys Chem C* 111:11320
29. Losito I, De Giglio E, Cioffi N, Malitesta C (2001) *J Mater Chem* 11:1812
30. Sayyah SM, El-Deeb MM, Kamal SM, Azooz RE (2009) *J Appl Polym Sci* 112:3695
31. Graf N, Yegen E, Gross T, Lippitz A, Weigel W, Krakert S, Terfort A, Unger WES (2009) *Surf Sci* 603:2849
32. Wu C-C, Chang H-C (2004) *Anal Chim Acta* 505:239
33. Kasik I, Mrazek J, Podrazky O, Seidl M, Aubrecht J, Tobiska P, Matejec V, Kovacs B, Markovics A, Szili M, Vlckova K (2008) *Mater Sci Eng C* 28:842
34. Yano J (1995) *J Polym Sci Part A: Polym Chem* 33:2435
35. Chiba K, Ohsaka T, Ohnuki Y, Oyama N (1987) *J Electroanal Chem* 219:117
36. Li X-G, Huang M-R, Duan W (2002) *Chem Rev* 102:2925
37. Cintra EP, Cordoba de Torresi SI, Errien N, Louarn G (2003) *Macromolecules* 36:2079
38. Szargan R, Schaufuss A, Rossbach P (1999) *J Electron Spectrosc Relat Phenom* 100:357
39. Zhao S, Shi Z-Q, Wang C-Y, Chen M-M (2008) *J Appl Polym Sci* 108:1852
40. Losito I, Malitesta C, De Bari I, Calvano C-D (2005) *Thin Solid Films* 473:104
41. Losito I, Palmisano F, Zambonin PG (2003) *Anal Chem* 75:4988
42. Xinman T, Qingji X, Meiling W, Youyu Z, Shouzhuo Y (2005) *Chin Sci Bull* 50:1598